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Examination of the Polymicrobial Interaction: Inhibitory Effects of Alcaligenes Species on Members of the Candida Species

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Examination of the Polymicrobial Interaction:

Inhibitory Effects of *Alcaligenes* Species on Members of the *Candida Species*

By

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Abstract

Candida species are commonly found in the human normal flora, however they are a major cause of nosocomial infections that can be life threatening. This fungal species is an opportunistic pathogen and causes infection in individuals who are immunosuppressed. A key characteristic of *Candida's* virulence is the ability to change its morphology from ovoid yeast to filamentous hyphae. *Alcaligenes* species are common bacteria found in the environment that rarely, if at all, cause infections in humans. It has been observed that when allowed to interact, *Alcaligenes faecalis* changes the morphology of *Candida albicans* from yeast cells to hyphal cells. When *A. faecalis* interacts with *Candida glabrata* or a mutated *C. albicans*, it causes no change in morphology and the cells are all in the yeast morphology. When allowed to interact with *C. albicans* and the mutated *C. albicans, Alcaligenes viscolactis* did not cause any changes in morphology to either type with all cells staying in the yeast form. Interestingly, however, both *A. faecalis* and *A.s viscolactis* both inhibited *C. albicans* and *C. glabrata* which was demonstrated on both agar plate interactions and liquid co-cultures. *A. faecalis* showed a greater inhibitory effect than *A.viscolactis.* The concentration of *A. faecalis* does not seem to be a contributing factor to the inhibitory effect it has on both *C. albicans* and *C. glabrata.* Taken together, these results demonstrate that *A. faecalis* and *A. viscolactis* could potentially be used to control one of the key virulence traits of *C. albicans* and could potentially identify new areas of study in Prokaryotic-Eukaryotic interactions, as well as, potential targets for treatment of *C. albicans* infections.

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Introduction

Overview:

Microbes are everywhere and are amazingly diverse. The human body is covered with microbes from the external skin to the internal lining of the digestive system where they serve many functions depending on where on the body the body they are found (10). Microbes can be an important advantageous part of the body, serving vital functions such as fighting infections and inhibiting pathogens (10). Some of the microbes that the human body is exposed to are pathogenic leading to infections. Some microbes are opportunistic pathogens which only cause problems when the human body is immunosuppressed and weak. Before antibiotics, bacterial infections were more often deadly leading to high mortality and morbidity rates. With the discovery and development of penicillin, healthcare completely changed. Antibiotics are an effective treatment for bacterial infections, but they must be used carefully. Antibiotics, like penicillin, have been used in excess to treat illness. Microbes are highly reactive to changes in the environment. They can easily and quickly evolve to thrive in new environmental conditions. Antibiotics can have a broad spectrum or narrow spectrum effect and when they are misused can have major consequences. The excessive use of broad spectrum antibiotics have led to high levels of antibiotic resistant bacteria and are progressively more difficult to treat. The rise of antibacterial resistant bacteria has caused researchers to attempt to understand alternative methods to inhibit pathogenic bacteria. Bacteria are living cells that require nutrients and space to survive, thus they constantly have to compete with other bacteria to thrive. Microbes interact with other microbes in both antagonistic and mutualistic ways, an area of which we know very little about.

***Candida* Species:**

***Candida albicans*:**

Candida albicans is a dimorphic fungus and a prevalent opportunistic pathogen, but is also commonly a part of the human microbiome (10). This fungi causes infections when individuals are immunosuppressed or when the immune system is compromised in some other way. *C. albicans* normally live in the gut, the genito-urinary tract, and the skin (4). Causing a variety of infections that can be mild like oral thrush, fungal urinary tract infections, genital yeast infections, or mucocutaneous candidiasis (13). Other infections that are caused by this fungi can be more serious and life threatening when it enters the bloodstream. These infections include candidemia, fungal endocarditis, endophthalmitis, fungal meningitis, and many others (13). This microbe is resistant to treatment through antifungals due to the many virulence factors it contains and to the biofilms it produces (4)(5). Biofilms are caused by microbial attachment to surfaces and to each other which makes them more likely to cause infection and increases resistance to treatment (6). This fungus is highly reactive to the environment which increases its resistance to antifungal treatments (3). This microbe also has been known to interact with different bacteria found on the body (10)(14). Due to the pathology of *C. albicans,* it is one of the most common causes of nosocomial infections. *C. albicans* transition between three different morphological forms which are yeast, pseudohyphal, and hyphal. It can form budding yeast which are round large cells. The fungi can undergo metamorphism and develop into Pseudohyphae which appears to be round cells with long branches sprouting from the cell. The hyphae form is a long spindle like cell. The virulence of *C. albicans* is connected to the morphological state of the fungi (12). The ability of *C. albicans* to transition between these morphological traits is key to its virulence as *C. albicans* that are locked into any one morphology lose their virulence potential.

***Candida glabrata*:**

Candida glabrata is similar to *C. albicans* in the fact that they are both commonly found in normal human flora and that they are both opportunistic pathogens (8). This fungi is found on the skin and in the body like the mouth and vagina (8). It can cause significant infections similar to *C. albicans* including life-threatening bloodstream infection (8). This fungi also produces biofilms which, like *C. albicans,* causes it to be more resistant to antifungal treatments (8). This microbe has key differences to *C. albicans. C. glabrata* is significantly smaller to *C. albicans* with *C. glabrata* being 1-4μm and *C. albicans* being 4-6μm (8). This fungus is not as virulent as *C. albicans* although it closely follows as the second most common cause of nosocomial fungal bloodstream infections (8). *C. glabrata* is different in morphology and stays in the budding yeast form (8). It does not develop into the hyphal morphology although under particular stress conditions it can develop into pseudohyphal (8). Another difference is that this fungus is not as reactive to the environment as *C. albicans* (7). *C. glabrata* is a haploid microorganism while *C. albicans* is diploid (8). The interaction between the human immune system and *C. glabrata* is not very well understood. However, *C. glabrata* interacts with macrophages interestingly as it can survive and continue to replicate inside of macrophages without causing damage to the immune cell (8). It is believed that macrophages can act as "trojan horses" for *C. glabrata* infections which increases its virulence (8). It is also understood that *C. glabrata* has innate azole resistance (8).

***Alcaligenes* Species:**

***Alcaligenes faecalis*:**

Alcaligenes faecalis is a bacteria that is commonly found in the soil and water (2). It is also commonly found in hospitals and the human body specifically in the normal flora of the

intestinal tract (15). This bacteria is Gram-negative bacillus and it is aerobic nonfermentative (15). *A. faecalis* is unusual as most Gram-negative bacteria are anaerobic (2). This microorganism is important ecologically as it has significant abilities to clean up after oil spills (2). It can also metabolize arsenite and change it into arsenate which is useful to neutralize contaminated environments (2). *A. faecalis* is an opportunistic pathogen and rarely causes infections (15). It has often been found from fluids associated with open wounds and in the ear (2). This bacteria can cause infection in individuals with compromised and suppressed immune systems. These infections are most often to occur in the hospital due to contaminated hospital equipment and body fluids (15). Although this bacteria has been found to cause infection in both humans and in birds, the virulent pathway of this bacteria is largely unknown (2).

***Alcaligenes viscolactis*:**

Alcaligenes viscolactis is extremely similar to *A. faecalis* as both are aerobic, nonfermentative bacilli. Like most members of *Alcaligenes*, this bacteria is also commonly found in the soil and in water (1). It is an opportunistic pathogen and rarely causes infection (1). *A. viscolactis* is found in milk and causes the milk to develop a ropy texture (9). This bacteria is also known to use amino acids, specifically L-Proline and L-Tyrosine, for growth and the production of slime (11). Not much more is known about this bacterium.

Methods

Microbial Strains and Culture Conditions. *C. albicans* and *C. glabrata* strains were maintained on Yeast Peptone Dextrose (YPD) agar plates and broth and grown at 37° C with shaking (250rpm) while A. faecalis and A. viscolactis were maintained on Luria Bertani (LB) agar and broth and grown at 37° C and 30° C respectively. For co-cultures, Brain Heart Infusion (BHI) broth and agar were used.

Quantitation of Morphological Changes. Candida and Alcaligenes strains were inoculated into their respective broth media and incubated at 37° C or 30° C overnight with shaking (250rpm). Overnight cultures were used as starter cultures to make mono and cocultures in the appropriate broth media by adding 50µl of the Candida species and 150µl of the Alcaligenes species. The mono and cocultures were then incubated for 4 hours at 37° C or 30° C**.** After the 4 hour incubation period, 20µl of the mono or the coculture were added to a microscope slide and observed using a Zeiss Primostar light microscope using the 100X objective. The number of yeast, pseudohyphal, and hyphal cells were counted from multiple representative fields on the microscope until the combined number reached 50 total cells.

Coculture Colony Forming Units (CFU) Candida and Alcaligenes strains were inoculated into their respective broth media and incubated at 37° C or 30° C overnight with shaking (250rpm). Overnight cultures were used as starter cultures to make mono and cocultures in the appropriate broth media. The optical density (OD600) of each starter culture was determined and used to equilibrate the amount of inoculum to use. Candida strains were inoculated to a concentration of ~$1x10^6$ cells/ml and Alcaligenes strains were inoculated to a concentration of ~$1x10^8$ cells/ml. The mono and coculture tubes were incubated for 24 hours with shaking at either 37° C or 30° C. Culture tubes were then serially diluted and plated on LB agar plates supplemented with 50µg/ml Kanamycin. LB agar plates were then incubated for an additional 24 hours, monitored for growth, and CFUs enumerated.

Zones of Inhibition Candida and Alcaligenes strains were inoculated into their respective broth media and incubated at 37°C or 30°C overnight with shaking (250rpm). Candida lawns were created on agar plates by taking a sterile Q-tip and spreading confluently over the surface of the plate. To four different microcentrifuge tubes, 1000µl of overnight cultures of Alcaligenes was added and centrifuged for five minutes at 10K rpm. The remaining liquid was decanted into a waste beaker, varying amounts of fresh LB broth was added (500µl, 250µl, 125 µl, and 65 µl), vortexed thoroughly to resuspend the bacterial cells, and 20µl of the new mixtures were spotted onto the *Candida* lawns. These agar plates were incubated at 37℃ or 30℃ for 24 hours. The following day, the zones of inhibition that had developed around the spots of Alcaligenes were measured.

Results

Alcaligenes faecalis causes a morphological change in *Candida albicans* cells

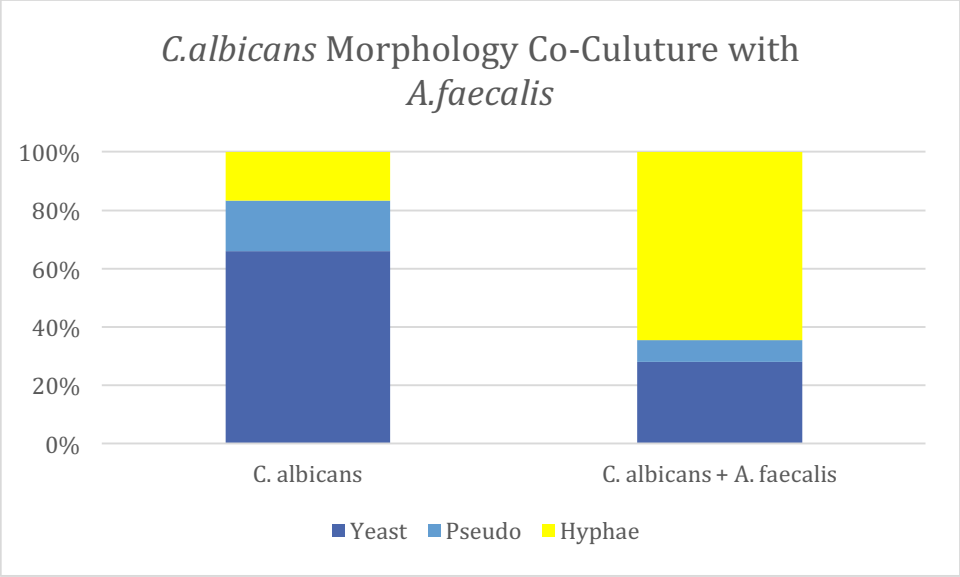
The yeast form of *C. albicans* is important for colonization and dissemination while the hyphal form of *C. albicans* is important for invasion and immune system evasion. Prior work in our laboratory has demonstrated that all three morphological forms of *C. albicans* are inhibited by *A. faecalis*, however *A. faecalis* inhibits the hyphal form at a higher rate than the other two forms. We therefor wanted to closely examine the morphological state of *C. albicans* when grown in monoculture verses co-culture with *Alcaligenes*. When *C. albicans* grew planktonically independent from any other bacteria, it grew primarily in the yeast form with minimal pseudohyphal or hyphal forms. When *C. albicans* was allowed to interact with *A. faecalis* in planktonic culture, it had a significant change in morphology from dominantly yeast form to primarily the hyphal form. This evidences that a major way *A. faecalis* could inhibit *C. albicans* is by causing it to change morphology to its hyphal form which may be more susceptible to the

physical interactions and inhibition that *A. faecalis* exerts upon it. In the control of pure *C. albicans*, it was observed that most of the *Candida* cells were in the yeast morphology (66%) with fewer cells in the pseudohyphal (17.4%) and hyphal (16.6%) form (Table 1 and Figure 1). In the co-culture of *C. albicans* and *A. faecalis,* there was significant morphological change in the C. *albicans* cells to predominantly hyphae (64.6%) rather than the pseudohyphal (7.4%) and yeast (28%) form, possibly due to the interaction with *A. faecalis* (Table 1 and Figure 1).

Table 1: Morphology *C. albicans* in monoculture or coculture with *A. faecalis*

	Yeast cells	Pseudo hyphal cells	Hyphal cells
Trial 1			
C. albicans	29	8	13
C. albicans + *A. faecalis*	10	6	34
Trial 2			
C. albicans	36	13	1
C. albicans + *A. faecalis*	11	3	36
Trial 3			
C. albicans	34	5	11
C. albicans + *A. faecalis*	21	2	27

Figure 1: The morphological state of *C. albicans* in monoculture and coculture with *A. faecalis*



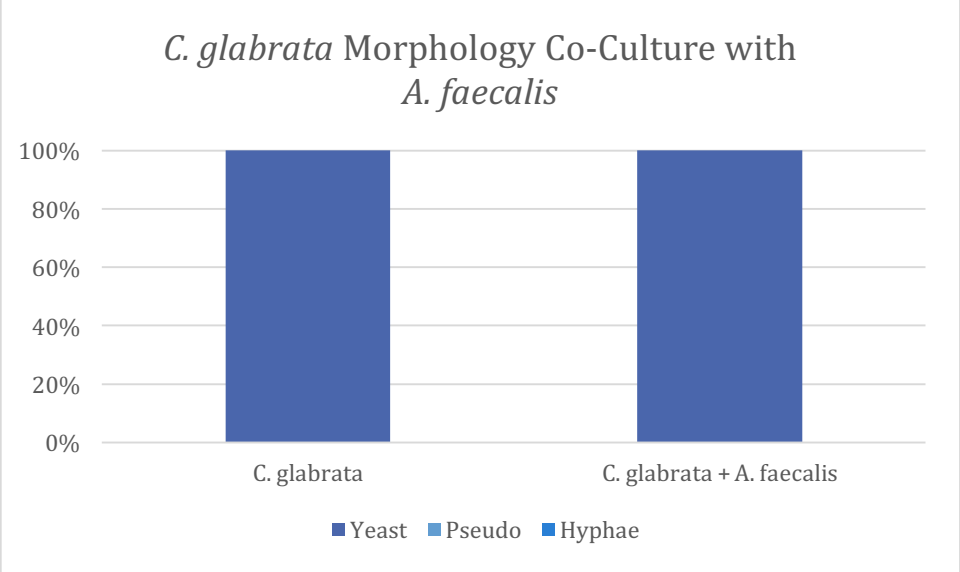
Alcaligenes faecalis* does not cause a morphological change in *Candida glabrata

As discussed above, *C. glabrata* is mainly restricted to yeast form and only changes due to extreme circumstances to the pseudohyphal form. We wanted to take the information from the *C. albicans/A. faecalis* coculture experiments to see if *A. faecalis* could promote the morphology of another *Candida* species. When *C. glabrata* is allowed to grow by itself, its morphology is exclusively in the yeast (100%) form with no pseudohyphal or hyphal forms (Table 2 and Figure 2). When this *C. glabrata* was mixed with *A. faecalis,* no morphological change was caused to the cells and they also exclusively remained in the yeast (100%) form (Table 2 and Figure 2). The cells stayed locked in yeast form. This suggests that although *A. faecalis* may have inhibitory effects on *C. glabrata*, it does not change the morphology of the cells or that morphology does not play a role in this inhibition. The inhibition of *C. glabrata* by *A. faecalis* does not appear to be due to changing the morphology or structures that are found on the different morphological forms.

Table 2: Morphology *C. glabrata* in monoculture or coculture with *A. faecalis*

	Yeast cells	Pseudo hyphal cells	Hyphal cells
Trial 1			
C. glabrata	50	0	0
C. glabrata + A. faecalis	50	0	0
Trial 2			
C. glabrata	50	0	0
C. glabrata + A. faecalis	50	0	0
Trial 3			
C. glabrata	50	0	0
C. glabrata + A. faecalis	50	0	0

Figure 2: The morphological state of *C. glabrata* in monoculture and coculture with *A. faecalis*



Candida* mutations in adhesion molecules resists morphological changes caused by *A. faecalis

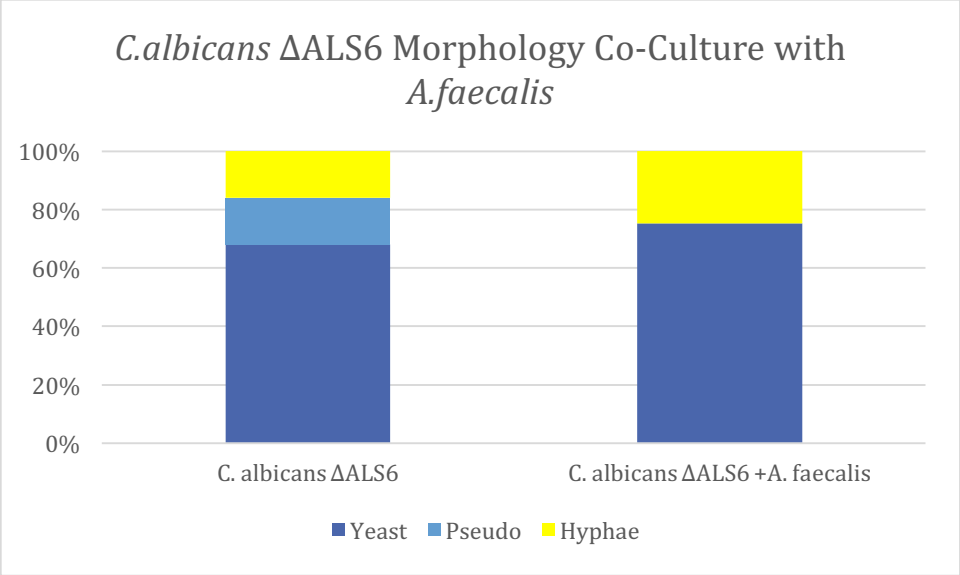
As discussed above, *A. faecalis* causes major morphological changes to *C. albicans.* We have previously shown in our lab that the inhibition *A. faecalis* exerts on *Candida* is through an unknown contact dependent mechanism as cell-free *A. faecalis* supernatant has no inhibition towards *Candida*. Recently, another laboratory has created strains of *C. albicans* mutated in Agglutin Like Sequences (ALS) that have been shown to be important in *C. albicans* interactions with both human cells and microbial cells. We therefore decided to test one of these mutant strains, ALS6, and observe how *C. albicans* morphology is affected. When *C. albicans* is mutated in the ALS6 gene, it does not respond to *A. faecalis* induced morphological changes as well. This suggests that when the *Candida* is mutated it becomes resistant to the inhibitory and morphological effects of *A. faecalis.* The *C. albicans* ΔALS6 monoculture had similar morphology arrangements as the wild-type *C. albicans* in previous experiments with predominantly yeast morphology (68%), followed by pseudohyphal and hyphal forms (both at 16%). Whereas, wild-type *C. albicans*, when grown with *A. faecalis,* shifted the morphology to hyphal form (68.4%) (Figure 1) the *C. albicans* ΔALS6 with *A. faecalis* has morphologies more similar to the monoculture with (74.6%) and hyphal (24.9%)(Table 3 and Figure 3). Interestingly, there was a lack of pseudohyphal forms in *C. albicans* ΔALS and *A. faecalis* co-culture.

Table 3: Morphology *C. albicans* ΔALS6 in monoculture or coculture with *A. faecalis*

	Yeast cells	Pseudohyphal cells	Hyphal cells
Trial 1			
C. albicans ΔALS6	37	0	13
C. albicans ΔALS6 + *A. faecalis*	50	0	0

Trial 2			
C. albicans ΔALS6	20	24	6
C. albicans ΔALS6 + *A. faecalis*	25	0	25
Trial 3			
C. albicans ΔALS6	45	0	5
C. albicans ΔALS6 + *A. faecalis*	37	0	12

Figure 3: The morphological state of *C. albicans* ΔALS6 in monoculture and coculture with *A. faecalis*



Alcaligenes viscolactis* does not cause a morphological change in *C. albicans

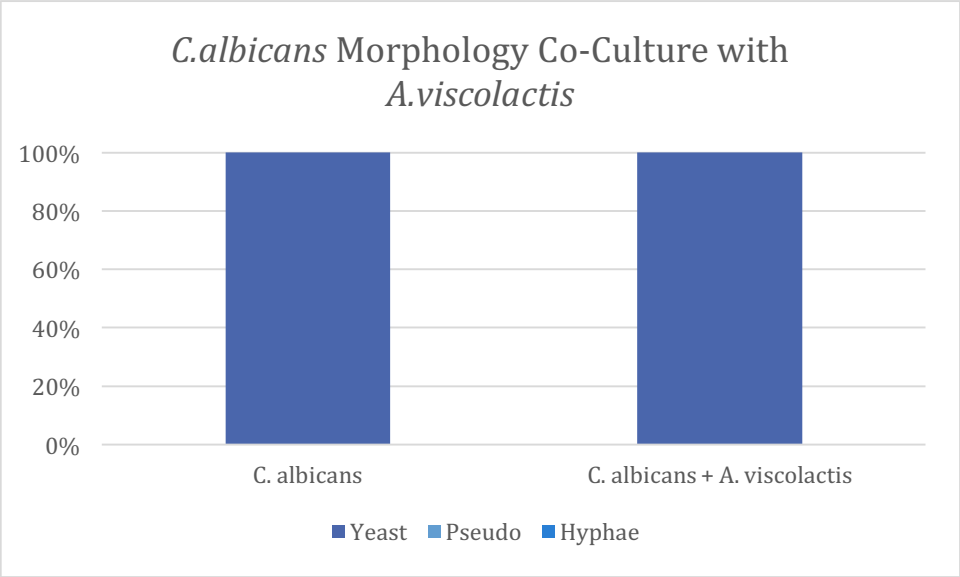
As we observed morphological changes due to the effects *A. faecalis* has on *C. albicans* and the *C. albicans* mutant, it is important to see if other species of *Alcaligenes* has a similar effect on Candida. Previous work in our laboratory has shown that *A. viscolactis* also inhibits *C. albicans* on agar and planktonic cultures. In both the monoculture of C. albicans and the coculture of C. albicans with A.viscolactis, all (100%) of the cells were in the yeast morphology (Table 4 and Figure 4). These experiments could suggest that *A. viscolactis* has a inhibitory effect on C. albicans growth, but does not have an effect on altering the morphology of *C.*

albicans. An important aspect that could have an effect on the morphology of *C. albicans* in this experiment is the growing conditions. While *C. albicans* grows optimally at both 37° C and 30° C, temperature can alter morphology of *C. albicans* with hyphal growth preferring the 37° C temperature and yeast morphology growth preferring 30° C. Additionally, *A. viscolactis* grows optimally at room temperature and is almost completely inhibited at 37° C. Thus, C. albicans may not produce any hyphae or pseudohyphae purely due to the temperature that is required for the co-culture with *A. viscolatis.*

Table 4: Morphology *C. albicans* in monoculture or coculture with *A.viscolactis*

	Yeast cells	Pseudo hyphal cells	Hyphal cells
Trial 1			
C. albicans	50	0	0
C. albicans + A.viscolactis	50	0	0
Trial 2			
C. albicans	50	0	0
C. albicans + A.viscolactis	50	0	0
Trial 3			
C. albicans	50	0	0
C. albicans + A.viscolactis	50	0	0

Figure 4: The morphological state of *C. albicans* in monoculture and coculture with *A. viscolactis*



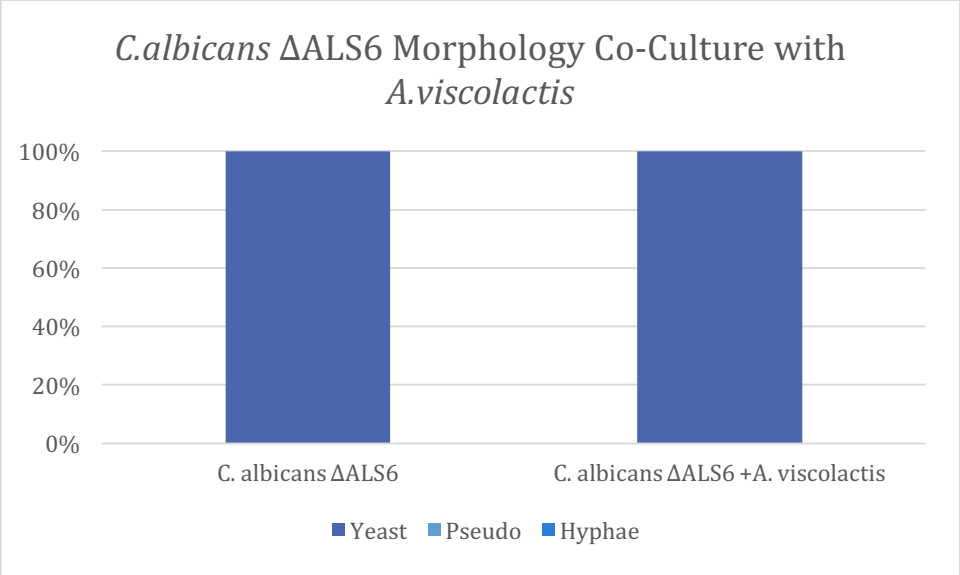
The observation that the lack of morphological change in *C. albicans* may be due to the temperature that is required for *A. viscolactis* seems to be supported when *C. albicans* ΔALS6 is grown in monoculture and coculture with *A. viscolactis*. In this experiment, all cells remain in the yeast form (100%) for both the control and experimental groups. Thus, our understanding of the morphology changes in *C. albicans* cannot be confirmed by *A. viscolactis* at this time.

Table 5: Morphology *C. albicans* ΔALS6 in monoculture or coculture with *A. viscolactis*

	Yeast cells	Pseudohyphal cells	Hyphal cells
Trial 1			
C. albicans ΔALS6	50	0	0
C. albicans ΔALS6 + *A.viscolactis*	50	0	0
Trial 2			
C. albicans ΔALS6	50	0	0
C. albicans ΔALS6 + *A.viscolactis*	50	0	0
Trial 3			

C. albicans ΔALS6	50	0	0
C. albicans ΔALS6 + *A.viscolactis*	50	0	0

Figure 5: The morphological state of *C. albicans* ΔALS6 in monoculture and coculture with *A. viscolactis*



***A. faecalis* significantly inhibits both *C. albicans* and *C. glabrata* growth in 24h planktonic cocultures as evidenced by colony forming units (CFUs).**

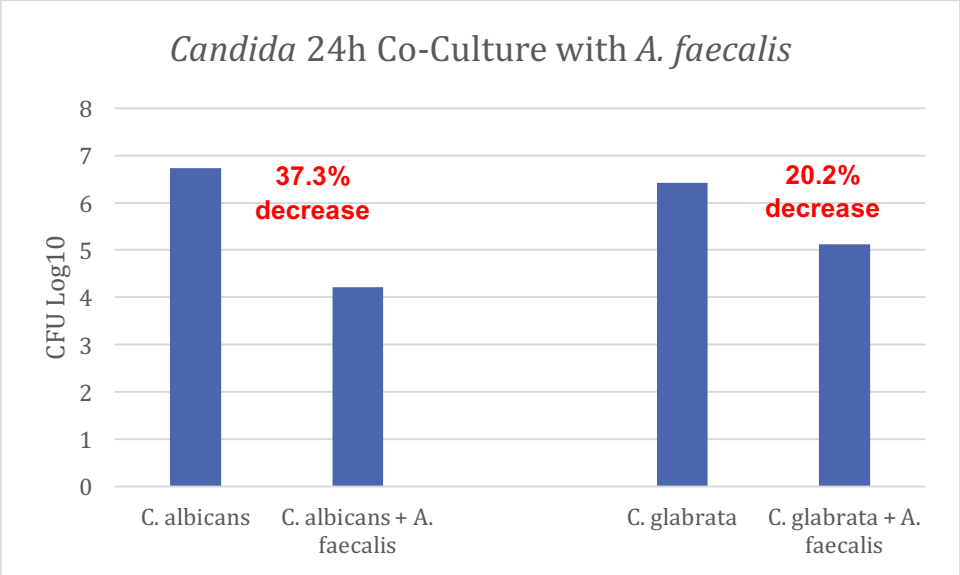
After demonstrating that *A. faecalis* has and effect on *C. albicans* morphology, we wanted to take the same microbial combinations and conditions to determine if *Alcaligenes* inhibited planktonic *Candida* growth. Using cocultures of *C. albicans* or *C. glabrata* with *A. faecalis* grown over 24 hours, we assessed the inhibitory effect of *A. faecalis* on the CFUs of *Candida*. There was a significant inhibition of *A. faecalis* on *C. albicans* planktonic growth. This inhibition reached an average of 37.3% fewer CFUs over 24 hours as compared to *C. albicans* monoculture controls (Table 6 and Figure 6). This growth inhibition was also observed in C. glabrata and A. faecalis co-cultures. The coculture of C. glabrata and A. faecalis produced a

smaller reduction, but was still able to reduce (20.2%) the CFUs over 24 hours (Table 6 and Figure 6).

Table 6: Growth inhibitory effects of *A. faecalis* on *C. albicans* and *C. glabrata* (CFUs)

	Plate #	Number of CFUs
Trial 1		
C. albicans	5	38
C. albicans + *A. faecalis*	3	56
C. glabrata	5	95
C. glabrata + *A. faecalis*	3	170
Trial 2		
C. albicans	5	75
C. albicans + *A. faecalis*	3	5
C. glabrata	5	53
C. glabrata + *A. faecalis*	3	136
Trial 3		
C. albicans	5	52
C. albicans + *A. faecalis*	3	17
C. glabrata	5	72
C. glabrata + *A. faecalis*	3	97

Figure 6: Growth inhibitory effects of *A. faecalis* on *Candida* (CFUs)



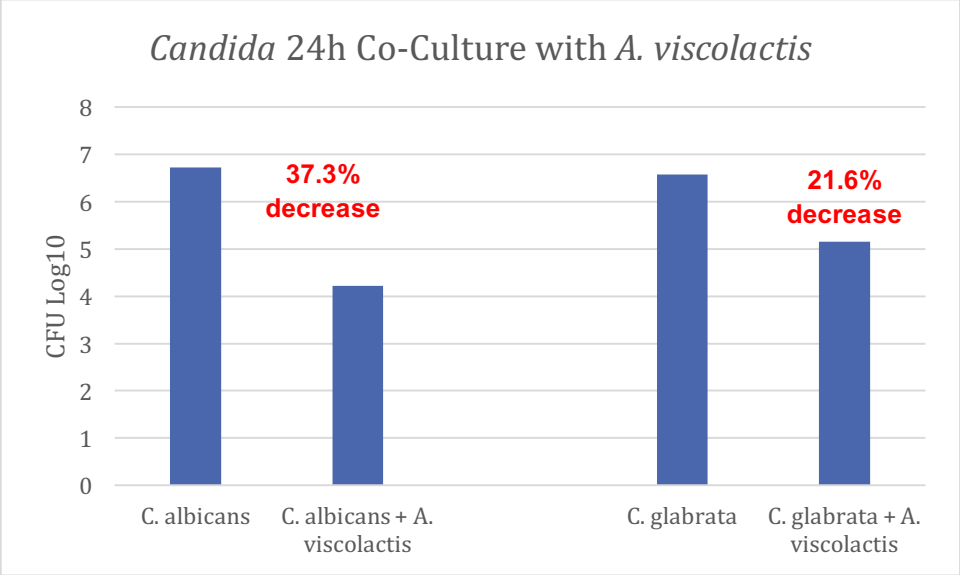
A. viscolactis significantly inhibits both C. albicans and C. glabrata growth in 24h planktonic cocultures as evidenced by colony forming units (CFUs).

Similar to the previous *A. faecalis* growth culture experiment, we wanted to see if the closely related *A. viscolactis* could also inhibit both *C. albicans* and *C. glabrata* growth. Table 7 and Figure 7 show the results of this set of experiments. As in the effects seen with *A. faecalis*, the coculture of *A. viscolactis* with *C. albicans* produced the largest reduction in CFUs with a decrease of 37.3%. Additionally, there was also a reduction of *C. glabrata* CFUs (21.6%), just not as large a decrease as with *C. albicans* (Table 7 and Figure 7). These results, in conjunction with the previous morphology, may show that morphology, particularly the hyphal form, is more susceptible to this inhibition. Since *C. glabrata* cannot produce hyphae, this may indicate why there is not as great a reduction in CFUs. However, we can see that although *A. viscolactis* cannot alter the morphology of *C. albicans*, most likely due to the temperature requirements, it still retains the ability to inhibit both *C. albicans* and *C. glabrata*.

Table 7: Growth inhibitory effects of *A. viscolactis* on *C. albicans* and *C. glabrata* (CFUs)

	Plate #	Number of CFUs
Trial 1		
C. albicans	5	57
C. albicans + A. viscolactis	3	76
C. glabrata	5	54
C. glabrata + A. viscolactis	3	126
Trial 2		
C. albicans	5	14
C. albicans + A. viscolactis	5	10
C. glabrata	5	38
C. glabrata + A. viscolactis	3	156
Trial 3		
C. albicans	5	10
C. albicans + A. viscolactis	3	102
C. glabrata	5	26
C. glabrata + A. viscolactis	3	147

Figure 7: Growth inhibitory effects of *A. viscolactis* on *Candida* (CFUs)



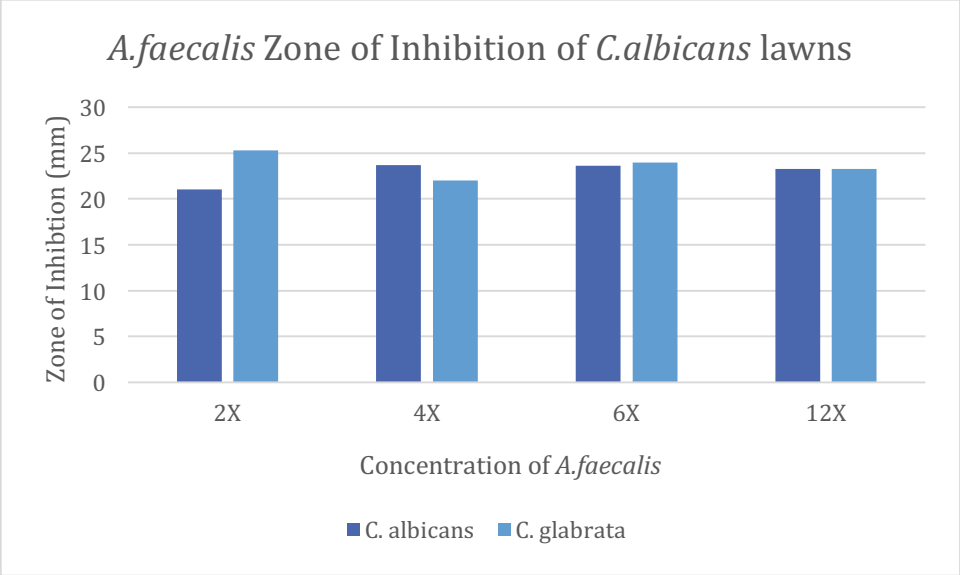
The concentration of *A. faecalis cells* does not have an effect on the amount of inhibition

We have been able to demonstrate that *A. faecalis* inhibits both *C.albicans* and *C. glabrata*, but whether the concentration or amount of *A. faecalis* cells needed to increase this inhibition is unknown. We attempted to determine if increasing the concentration of *A. faecalis* cells involved in the interaction will increase the amount of *C. albicans* inhibition by spotting varying increasing concentrations of *A. faecalis* on lawns of *C. albicans* and measuring the zones of inhibition (ZOI). To do this, we used the same overnight cultures of A. faecalis, made 1000µl aliquots, centrifuged the cells and resuspended them in a reduced volume creating 2X, 4X, 8X, and 12X concentrations. These concentrations were spotted onto Candida lawns on agar plates, incubated for 24 hours and the ZOI measure. Table 8 and Figure 8 show the results of this inhibition. After a certain point (4X) there does not seem to be much effect on the amount of inhibition seen on *C. albicans* or *C. glabrata*. There is a small increase in ZOI from 2X to 4X, but after the 4X concentration, the ZOIs remain the same between the varying concentrations.

Table 8: *A. faecalis* Zones of Inhibition on *Candida* lawns (millimeters)

Zones of Inhibition					
		2X *A.faecalis*	4X *A.faecalis*	8X *A.faecalis*	12X *A.faecalis*
Trial 1	*C. albicans*	25mm	23mm	27mm	22mm
	C. glabrata	26mm	21mm	27mm	24mm
Trial 2	*C. albicans*	16mm	20mm	22mm	23mm
	C. glabrata	20mm	21mm	22mm	21mm
Trial 3	*C. albicans*	22mm	28mm	22mm	25mm
	C. glabrata	30mm	24mm	23mm	25mm

Figure 8: *A. faecalis* Zones of Inhibition on *Candida* lawns (millimeters)



Discussion

The *Alcaligenes* species, which is commonly found in the water and soil, has inhibitory effects on *Candida* species. The level of inhibition varies depending on the interaction between specific combinations of Alcaligenes species and Candida species with the combination of *A.*

faecalis showing the greatest level of inhibition on *C .albicans.* The interaction between these two microbes causes a morphological change in *C. albicans* from the typical yeast cell to the hyphal cell morphology. This observation calls into question if the method of inhibition of *A. faecalis* on *C. albicans* is the changes in morphology. The concentration of *A. faecalis* did not have a significant impact on the inhibition it causes to *C. albicans*. When *Candida* is mutated, particularly in the ALS6 sequence, it seems to resist the morphological changes and stays mostly in yeast form. There was no morphological change, however, observed in the interaction between *A. faecalis* and *C. glabrata.* Although not as high of a reduction, inhibition of *C. glabrata* by *A. faecalis* did occur. As there were no changes in the morphology of *C. glabrata,* there is no argument for changes in morphology being the method of inhibition by *A. faecalis.* The concentration of *A.s faecalis* also had no significant impact on the degree of inhibition observed on *C. glabrata,* but *A. viscolactis* has inhibitory effects on both *Candida albicans* and *Candida glabrata.* There is no observable change in morphology of the *C. albicans* when *A. viscolactis* interacts with *C. albicans.* The same observation was made when *A. viscolactis* interacted with mutated *C. albicans* in the ALS6 gene. The optimal growing conditions for *Candida* species is at 37℃, but *A. viscolactis*, however, grows optimally at room temperature. This difference in growing conditions could be a factor that affects the results of these experiments.

The future of this project would be to look at the genetics of *Alcaligenes* and *Candida* species and to determine how the inhibition from the *Alcaligenes* species occurs. Next steps could include pinpointing the genes in *C. albicans* involved in this interaction with *A. faecalis.* Another aspect to further research would be how *Alcaligenes* species interact with different C. albicans mutated in the ALS family. To date, there are nine different ALS genes (ALS1-ALS9)

that C. albicans possesses. Determining if all, none, or specific combinations of these genes are essential in the inhibition of Alcaligenes with Candida.

The clinical status of treatment of bacterial and fungal infections is problematic in the way that microbes easily evolve to be resistant to current antibiotics and antifungals. The misuse of antibiotics led to the development of major pathogens like Methicillin resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*. In the beginning of antibiotic treatment, there were hundreds of antibiotics that could be used and were effective, that number has decreased massively and continues to dwindle. It is important, if not vital, to find alternative treatments for bacterial and fungal infections. This project is one of hundreds that are hoping to make the first steps in the direction of discovering an alternative treatment method. This project looks specifically at using one microbe to inhibit another. The hope is that projects like this one can be the stair-steps in discovering alternative treatment methods.

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